TECHNICAL MANUSCRIPT 190

EFFECT OF MORTALITY-ENHANCING FACTOR FROM LISTERIA ON EXPERIMENTAL HISTOPLASMOSIS

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EFFECT OF MORTALITY-ENHANCING FACTOR FROM LISTERIA ON EXPERIMENTAL HISTOPLASMOSIS

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ABSTRACT

A crude, heat-stable cell extract of Listeria monocytogenes that increases susceptibility of laboratory animals to infections with a variety of bacteria has been previously reported. This preparation was labeled mortality-enhancing factor (MEF).

This report describes investigations to determine the effect of MEF on experimental histoplasmosis, principally in Syrian hamsters. In a preliminary study, yeast cells of Histoplasma capsulatum Scritchfield strain were injected intraperitoneally with and without serial dilutions of MEF into mice and hamsters. Most of the animals that received MEF and yeast cells died within 15 days postinoculation.

In further experiments to determine minimal amounts of MEF required to increase susceptibility to infection, microconidia from strains No. 5 and Ross were employed. Time to death was reduced from 6 or 9 weeks when strains No. 5 and Ross were injected intraperitoneally; to less than 1 week when the maximum MEF, 14.5 mg per animal, was administered with spores. Once the animals started to die, however, the rate of death and total numbers of dead animals were approximately the same as those for control animals receiving spores alone.

EFFECT OF MORTALITY-ENHANCING FACTOR FROM LISTERIA ON EXPERIMENTAL HISTOPLASMOSIS

Silverman, Elwell, and Kautter[1] have previously demonstrated a crude, heat-stable cell extract of Listeria monocytogenes that increased the susceptibility of laboratory animals to infections with a wide variety of bacteria. This extract was prepared by centrifuging suspensions of sonically disrupted cells at 25,000 x g and filtering the supernatant fluid through sintered glass filters of ultrafine porosity. The preparation was labeled mortality-enhancing factor (MEF), and until use was stored at -50 C. The susceptibility of mice,* guinea pigs, rabbits, and rhesus monkeys to infection with listeria was increased when MEF was used prior to, at the same time, or subsequent to the injection of infecting organisms. It was also effective when administered by routes other than that used for inoculation. The cell extract also rendered mice more susceptible to a number of other gram-positive and gram-negative bacteria. The chemical nature of MEF and much of its mode of action on the host remains to be determined; however, it has been shown to have no effect on phagocytosis either in vivo by the leucocytes of induced peritoneal exudates or in vitro by the polymorphonuclear leucocytes of heparinized blood. Changes in serum components such as lysozyme, complement, and properdin suggest that one possible mode of action may be decreased bactericidal activity following injection of MEF. A rapid increase of listeria in spleens and livers of mice that received both cells and extract when compared with control animals could indicate a possible interference by MEF with the clearance mechanism of the host.

This report describes the effects of crude MEF prepared in our laboratory on experimental histoplasmosis, principally in Syrian hamsters.

A preliminary study was conducted with the yeast phase of Histoplasma capsulatum Scritchfield strain obtained from Dr. Furcolow.** Four-day-old yeast cells grown in Salvin's liquid medium were injected intraperitoneally with and without MEF into mice and hamsters. The MEF employed in these experiments had a dry weight of 43.6 mg/ml. Serial dilutions that contained from 0.45 to 14.53 mg of MEF were administered to each

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1. Silverman, S.J., L. Elwell, and D.A. Kautter. 1961. A mortality enhancing extract isolated from Listeria monocytogenes. 86:669-674.

* In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

** Dr. M.L. Furcolow, Dept. of Microbiology, University of Kentucky, Lexington, Kentucky.

animal along with 2.8×10^7 yeast cells. Control animals received dilutions of yeast cells only, since previous investigations had shown MEF to be innocuous. The experiment was terminated after 15 days. At that time most of the animals that received yeast cells and MEF were dead, as shown in Table 1, while only a few of the control animals that had been injected with yeast cells alone died. These results indicated that the inclusion of MEF in yeast-cell inoculum shortened time to death, but to an unknown degree, because an extension of the experiment beyond 15 days might have allowed additional control animals to expire. In addition, the minimal amount of MEF necessary to produce such an effect was not determined.

TABLE 1. EFFECT OF MORTALITY-ENHANCING FACTOR (MEF) DERIVED FROM LISTERIA MONOCYTOGENES ON HAMSTERS INJECTED INTRAPERITONEALLY WITH HISTOPLASMA CAPSULATUM SCRITCHFIELD STRAIN

MEF, mg	Yeast Cells Injected	No. Dead/No. Injected	
		Mice	Hamsters
14.53	2.8×10^7	10/10	9/10
7.26	2.8×10^7	10/10	9/10
3.63	2.8×10^7	10/10	7/10
1.81	2.8×10^7	10/10	6/10
0.91	2.8×10^7	6/10	10/10
0.45	2.8×10^7	6/10	8/10
0	8.1×10^7	7/10	3/10
0	2.8×10^7	3/10	0/10
0	8.1×10^6	1/10	1/10
0	8.1×10^5	0/10	0/10
0	8.1×10^4	0/10	0/10

Experiments to establish an endpoint for the MEF effect on experimental infection with microconidia of the mycelial phase were conducted. H. capsulatum strain 5, obtained from Dr. Jan Schwarz,* and a Ross strain

* Dr. Jan Schwarz, 1224 Westminster Drive, Cincinatti, Ohio.

furnished by Dr. S.K. Sinha,* were grown on the surface of BBL Mycophil** agar in 250-ml Erlenmeyer flasks at room temperature for 3 weeks. Microconidia spores were removed from agar surfaces by agitation with buffered saline and glass beads. Total counts of spore suspensions thus prepared were made in hemacytometers and viable numbers were determined by spreading appropriate dilutions on blood agar plates. Nine groups of 10 hamsters each were injected intraperitoneally with serial dilutions of MEF ranging from 0.11 to 14.5 mg per animal for each of the two histoplasma strains tested. Doses of 2×10^6 or 5×10^6 viable spores of the Ross or No. 5 strains, respectively, were injected with the MEF preparation.

Liver and spleen slices of expired experimentally infected hamsters were smeared on blood plates containing penicillin, dihydrostreptomycin, and actidione to inhibit bacteria and saprophytic fungi. Except in a few instances where intestinal flora contaminated the blood plates, H. capsulatum was recovered from nearly all test animals. Time to death was reduced from 6 or 9 weeks when no MEF was administered, to less than 1 week when the maximum MEF, 14.5 mg per animal, was administered with the spores; however, rates of death and total numbers of dead animals were approximately the same as those found for control animals receiving spores alone, as shown in Figure 1. A gradual increase in time to death with diminishing MEF was found for both histoplasma strains tested. Figure 2 indicates that by two months postinoculation with H. capsulatum strain No. 5, almost all animals were dead. An approximate titration of the amount of MEF required to bring about altered animal death patterns within a one-month period may be seen. Figure 3 shows similar results when hamsters were inoculated with the Ross strain of H. capsulatum, except that most of the animals were dead 4 months postinoculation. The high, cumulative per cent mortality of hamsters injected with strain No. 5 and MEF was concluded in 2 months; that of animals injected with the Ross strain and MEF required 4 months. It is not evident from the data presented whether the variation in host response is due to differences in strain virulence, or to the fact that two and one-half times as many spores of strain No. 5 were administered per animal.

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* Dr. S.K. Sinha, Central Wisconsin Colony and Training School, Madison 4, Wisconsin.

** Baltimore Biological Laboratories.

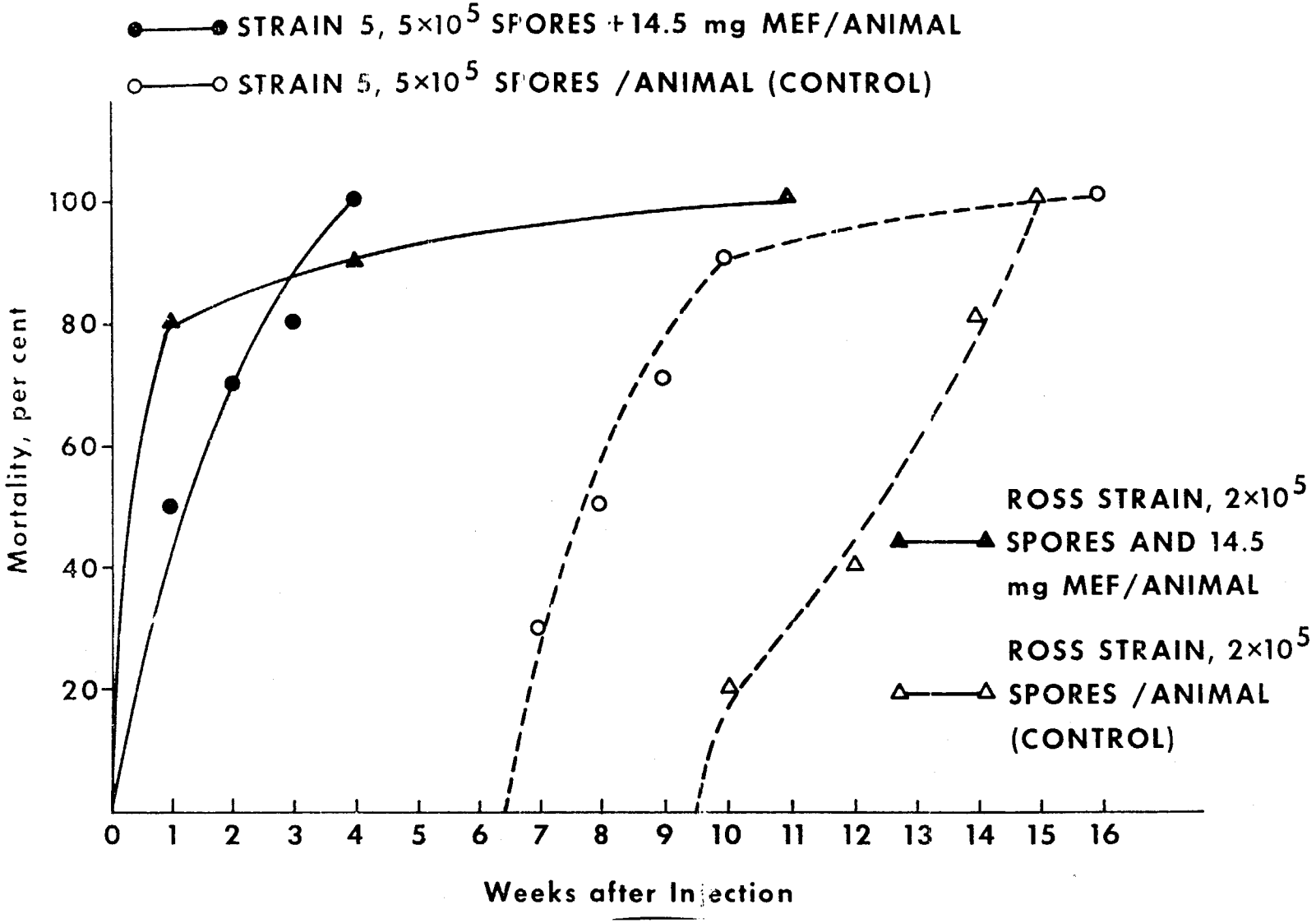


Figure 1. Effect of Mortality-Enhancing Factor (MEF) Derived from Listeria monocytogenes on Experimental Histoplasmosis in Hamsters.

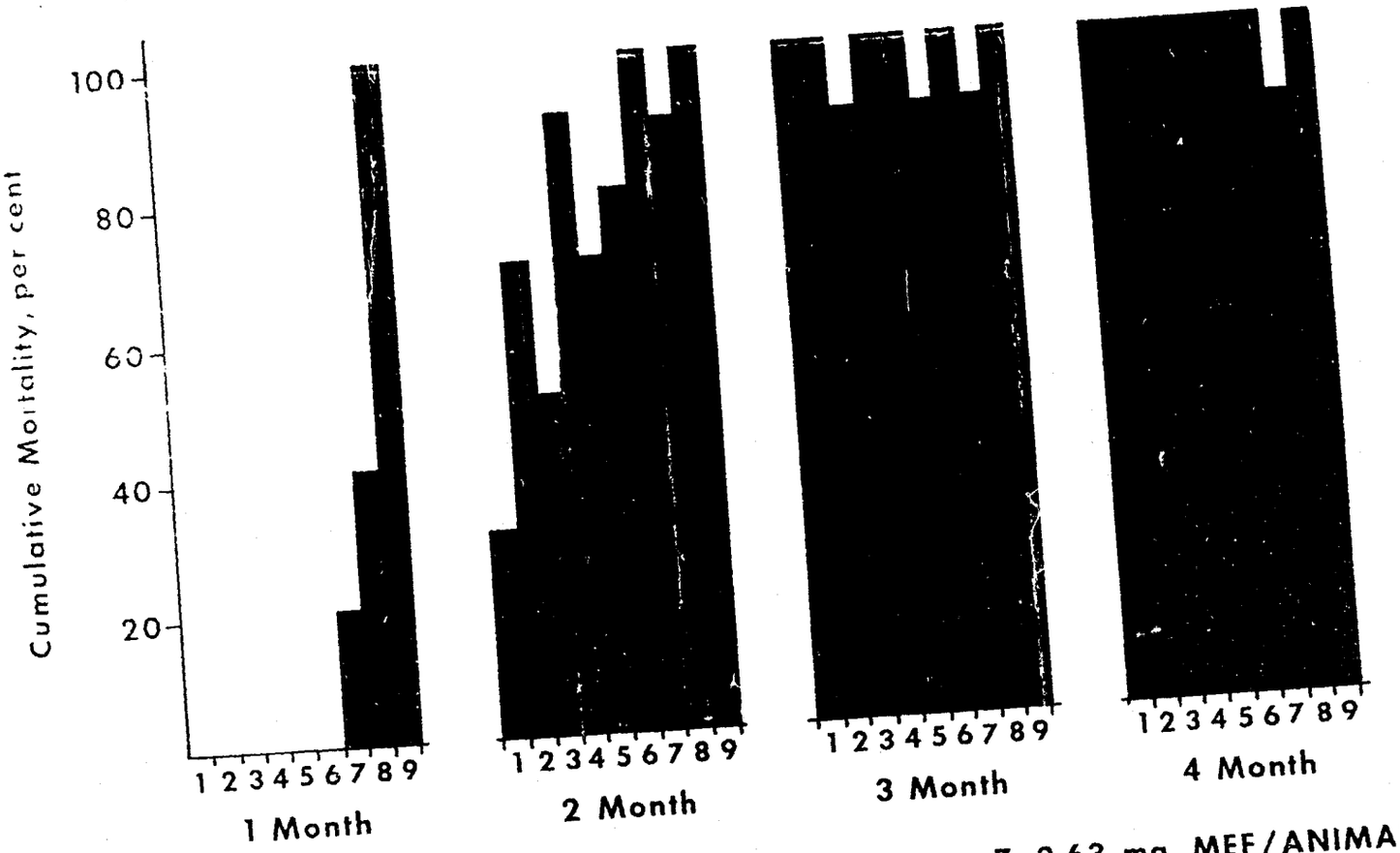
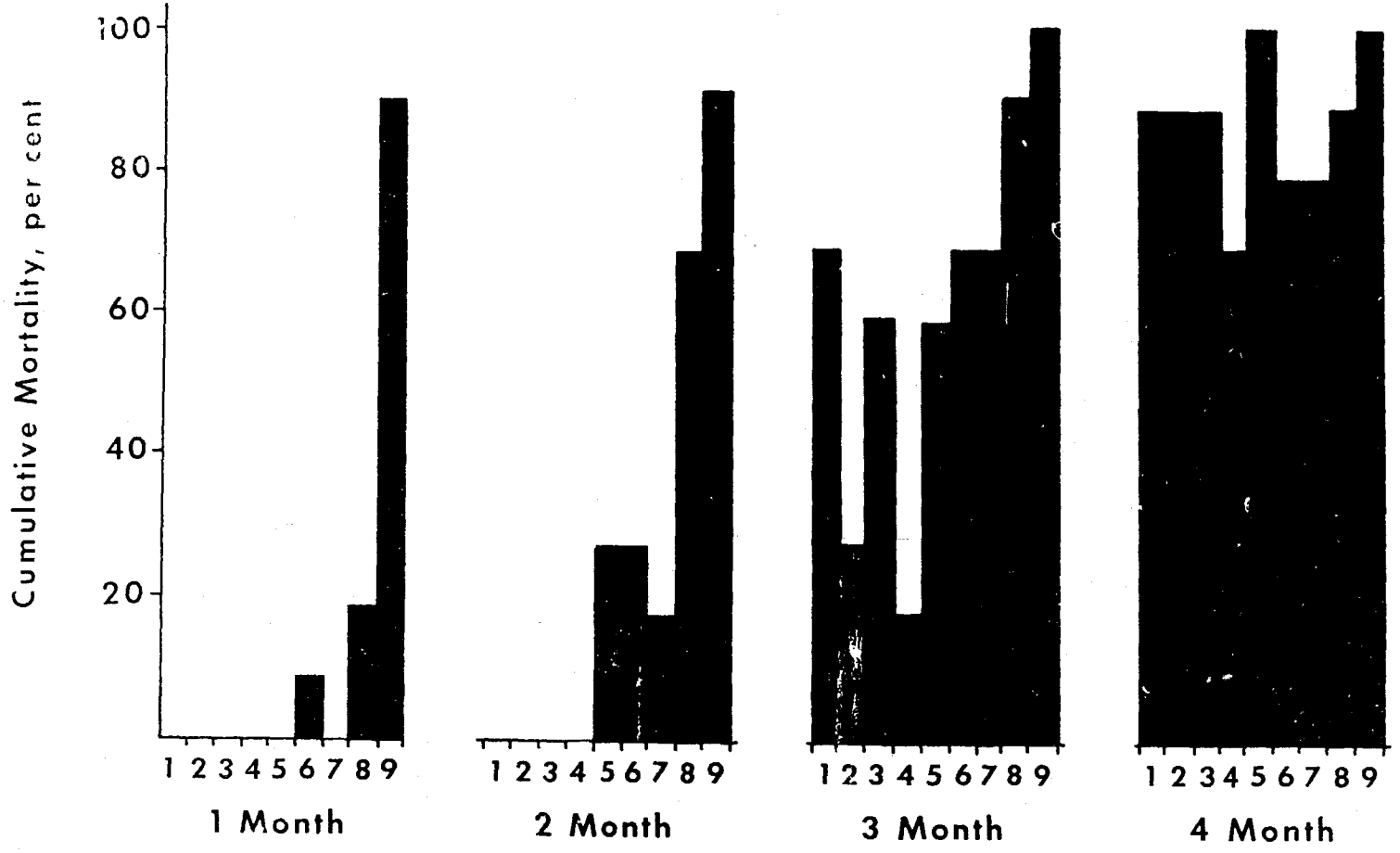


Figure 2. Effect of Mortality-Enhancing Factor (MEF) Derived from *Listeria monocytogenes* on Hamsters Injected Intraperitoneally with *Histoplasma capsulatum* Strain No. 5.[a]

a. All animals received 5.0×10^5 spores



1. SPORES ALONE
2. 0.11 mg. MEF/ANIMAL
3. 0.23 mg. MEF/ANIMAL
4. 0.45 mg. MEF/ANIMAL
5. 0.91 mg. MEF/ANIMAL
6. 1.81 mg. MEF/ANIMAL
7. 3.63 mg. MEF/ANIMAL
8. 7.26 mg. MEF/ANIMAL
9. 14.53 mg. MEF/ANIMAL

Figure 3. Effect of Mortality-Enhancing Factor (MEF) Derived from *Listeria monocytogenes* on Hamsters Injected Intraperitoneally with *Histoplasma capsulatum*, Ross Strain.[a]

a. All animals received 2.0×10^5 spores

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